

ORIGINAL ARTICLES

Preparation of Zinc-Rich Powder from Oysters and Evaluation of Its Bioavailability

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Abstract

Hot water extract of oyster is used as a raw material of several nutritional supplements and oyster sauce. However, most of zinc in oysters is difficult to be recovered by the hot water extract method. In this study, we examined a zinc-rich powder fraction (ZRP) from oyster scraps in preparation of the oyster extract, and evaluated its bioavailability by animal assay and an *in vitro* digestion test. In preparation of ZRP, extraction with 0.1M HCl was performed for the oyster scraps. After neutralization of the acid extract, the precipitation was collected, freeze-dried and used as ZRP. The zinc content of ZRP was 76 mg/g in the dry base. About 70% of the zinc in the scraps was recovered into ZRP. In the evaluation of bioavailability, male weanling Wistar rats were pair-fed a low zinc-based diet (zinc content, 1.41 $\mu\text{g/g}$) or a basal diet supplemented with zinc carbonate hydroxide or ZRP (zinc content, 5 $\mu\text{g/g}$) for 4 weeks. The zinc content of the tibia of the rats supplemented with ZRP was significantly higher than that of the rats supplemented with zinc carbonate hydroxide. In an *in vitro* digestion test, when the zinc-supplemented diet was digested with trypsin, the zinc in ZRP was solubilized more rapidly than that in zinc carbonate hydroxide. These results indicate that ZRP contains a high level of zinc in a highly available form. Accordingly, ZRP can be used as a zinc supplement foodstuff.

Key words: zinc, oyster, bioavailability, zinc supplement foodstuff.

INTRODUCTION

Zinc is an essential trace element in human nutrition. The latest National Nutritional Survey indicated that there is a sub-optimal zinc level in the Japanese population [1]. To improve zinc nutrition, the effective utilization of several food sources containing a high zinc concentration is necessary. According to the Standard Tables of Food Composition in Japan, oysters contain zinc at a particularly high level [2], and so their use is expected in the zinc enrichment of foods or the production of the zinc supplements.

In Japan, hot water extract of oyster (OE) is utilized as a raw material of several supplements and oyster sauce. However, much of the zinc in oysters is insoluble in water at a neutral pH [3], and cannot be extracted with hot water. This means that the zinc concentration in OE is too low to use as a zinc supplement. Although most of the zinc remains in the oyster after hot water extraction, the oyster is scrapped as waste without recovering the zinc.

In this study, we used the oysters scrapped in OE production to recover zinc as a form of zinc-rich powder (ZRP). In addition, we studied the bioavailability of zinc in the powder by *in vivo* animal assay and *in vitro* digestion test.

MATERIALS AND METHODS

Samples. Hot air-dried samples of oysters scrapped

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in OE production were kindly supplied by Japan Clinic Co. (Kyoto, Japan) and ground using a mill (Grindomix GM200, Retsch GmbH & Co, Haan, Germany). Analysis using an atomic absorption flame emission spectrophotometer (AA-6200, Shimadzu, Kyoto, Japan) showed that the zinc content in the samples was 3.07 mg/g.

Preparation of ZRP. The dried powder of the oyster scraps was mixed with 20 volumes of 0.1 M HCl. The mixture remained at room temperature with gently shaking for 16 h, and was then centrifuged at 750 x g for 20 min. The supernatant obtained was adjusted to pH 7.0 by the dropwise addition of 1.0 M NaOH. The precipitation formed after neutralization was collected by centrifugation at 6,000 x g for 20 min. The precipitation was freeze-dried and used as ZRP from oysters.

Animal feeding. The experimental protocol was reviewed and approved by the Animal Ethics Committee of Kansai Medical University and followed the Guide for the Care and Use of Experimental Animals of the Prime Minister's Office of Japan. Four-week-old male Wistar rats (n=18), weighing 40 to 50 g each, were housed in stainless steel wire mesh hanging cages at a temperature of 22 to 24 °C and a humidity of 60% in a room with a controlled 12 h light (08:00 to 20:00) and dark cycle. The rats were given deionized water *ad libitum* and pair-fed the diet throughout the experimental period.

They were randomly divided into 3 groups. One group was fed a casein-based low zinc diet (referred to as the basal diet). The other two groups were fed the experimental diets, supplementing the basal diet with 5 µg/g of zinc as either zinc carbonate hydroxide or ZRP. The composition of the basal diet was (%): casein, 20.0; sucrose, 15.0; α -starch, 52.0; soybean oil, 8.0; AIN93G mineral mixture (except for zinc carbonate hydroxide), 3.5; AIN93G vitamin mixture, 1.0; choline bitartrate, 0.2; DL-methionine, 0.3; and cellulose powder, 2.0. Analysis showed that the basal diet contained 1.41 µg/g of zinc. After feeding for 4 weeks, the blood, liver, muscles and tibia were isolated, washed, blotted and weighed. The Blood was collected by heart puncture under the anesthetizing with diethylether.

In vitro digestion. Ten grams of each zinc-supplemented diet were mixed with 50 ml of 0.1N HCl containing 300 mg of a dried powder of porcine pepsin (1:10,000, Wako Pure Chemical Industries, Osaka) and incubated with shaking at 37 °C for 3 h. The pH of this reaction mixture was 1.3. After pepsin digestion, the pH of the reaction mixture was adjusted to 7.4 by the dropwise addition of 1M NaOH. Then, 30 mg of crystalline porcine trypsin

(5,600 USP trypsin unit/mg, Wako) was added to the mixture and incubated with shaking at 37 °C for a further 16 h. At each step of the *in vitro* protease digestion, a portion of the reaction mixture was centrifuged at 6,000 x g for 30 min.

Zinc in each soluble fraction obtained was directly determined with an atomic absorption flame emission spectrophotometer and the solubility was estimated as a percent of the contents of the soluble fraction to that in the whole mixture.

Assays. The chemical composition of ZRP was analyzed as follows. Moisture was measured by oven drying at 105 °C. Crude protein content was calculated from Kjeldahl nitrogen. Crude lipid was extracted by diethylether with a Soxhlet extractor after a hydrolysis with 35% HCl, and then weighed. Ash was weighed after direct incineration at 550 °C. After a simultaneous digestion with α -amylase, amyloglucosidase and protease, the undigestible fraction formed was collected, dried and incinerated. Dietary fiber in the ZRP was calculated as a difference between the dry weight and ash content of the undigestible fraction as described by Prosky [4]. Carbohydrate content was calculated from the following equation: 100 - (moisture + crude protein + crude lipid + ash + dietary fiber). Glycogen was extracted with hot water and determined with anthrone [5].

The ash resulting from the direct incineration of ZRP was dissolved in 1% HCl and subjected to mineral analysis. Sodium, potassium, magnesium, zinc, copper and manganese levels were determined using an atomic absorption flame emission spectrophotometer [6]. Phosphorous was determined colorimetrically using molybdate and vanadate [6]. Iron was determined with 1,10-phenanthroline chloride [6]. Calcium was determined by titration using oxalic acid and potassium permanganate [6].

The rat tissue was mixed with HNO₃ and heated in a boiling water bath until the insoluble components disappeared. The acid digests were then diluted with deionized water. The zinc level in the diluted acid digests was determined with an atomic absorption flame emission spectrophotometer. The zinc analysis was verified using standard reference materials (RM 8414, bovine muscle powder, National Institute of Standard & Technology, USA).

Statistics. Data obtained from the animal experiments were assessed by analysis of variance (ANOVA) followed by a PLSD test for multiple comparisons using a personal computer (eMac, Apple Computer, Cupertino, CA, USA).

with the statistical analysis software package StatView ver. 5.0 (Abacus Concepts, Berkeley, CA, USA).

RESULTS

Recovery of zinc. More than 95% of the zinc in the oyster scraps was extracted by 0.1 M HCl. Neutralization of the extract with NaOH formed a precipitate and 70 to 80% of the zinc extracted was recovered in the precipitate. Thus, about 70% of the zinc in the oyster scraps was recovered in the precipitate.

Chemical composition. Freeze-dried powder of the precipitate, that is, the oyster ZRP, was gray in color with a slight fishy odor. Table 1 shows the major components of ZRP, which was mainly composed of Kjeldahl nitrogen and ash.

Table 2 describes several minerals in ZRP, which con-

tained zinc at a level of 76 mg/g, 25 times higher than the original oyster scraps. The levels of sodium, calcium and phosphorous were comparable to zinc, but other minerals were less than 10 mg/g.

In vitro digestion. Table 3 shows the ratio of soluble zinc in each step of the *in vitro* protease digestion of the zinc-supplemented diets. At a neutral pH, the zinc solubility of the experimental diets was less than 30%, irrespective of the zinc source. Most of the dietary zinc was solubilized by 0.1 M HCl regardless of pepsin digestion, but was re-precipitated by neutralization.

Figure 1 shows the release of soluble zinc from each zinc-supplemented diet during trypsin digestion. The zinc in both diets was completely solubilized by trypsin digestion for 16 h. However, the speed of release varied with the zinc source, that is, the zinc in ZRP was more

Table 1. Composition of zinc-rich powder of oysters

	Content (%)
Moisture	2.7
Crude protein	43.4
Crude lipids	1.2
Ash	40.1
Dietary fiber	1.5
Carbohydrate	11.1
Glycogen	6.1

Table 2. Mineral content of zinc-rich powder of oysters

Minerals	Content (mg/g)
Zinc	76.0
Sodium	62.5
Phosphorus	46.5
Iron	4.70
Calcium	22.4
Potassium	4.00
Magnesium	4.05
Copper	2.78
Manganese	1.26

Table 3. Solubility of zinc contained in experimental diets

Solvent or treatment	Solubility of zinc added (%) ¹⁾	
	Zinc carbonate hydroxide	Zinc-rich powder of oysters
Water ²⁾	7.6±2.4	7.0±2.6
0.1M NaCl ²⁾	13.3±3.1	14.4±4.6
50 mM, Tris-HCl buffer (pH 7.4) ²⁾	27.1±3.5	23.3±4.5
0.1 M HCl ²⁾	89.4±5.9	91.6±4.7
Pepsin digest (pH 1.3) ³⁾	89.1±6.1	92.4±3.8
Pepsin digest (pH 7.4) ³⁾	17.5±4.5	21.3±5.1

¹⁾ Values are means ± SD for 4 trials. Significant difference (p<0.05) was not observed between zinc carbonate hydroxide and zinc-rich powder of oysters in all the solvents or treatments.

²⁾ Ten grams of each zinc-supplemented diet were mixed with 50 ml of each solvent.

³⁾ Ten grams of each zinc-supplemented diet were mixed with 0.1N HCl containing 300 mg of a dried powder of porcine pepsin and incubated with shaking at 37 °C for 3 h. The pH of this reaction mixture was 1.3. After pepsin digestion, the pH of the reaction mixture was adjusted to 7.4 by the dropwise addition of 1M NaOH.

rapidly solubilized than that in zinc carbonate hydroxide. At 1 h, the ratio of soluble zinc from ZRP was more than 80%, while that from zinc carbonate hydroxide was less than 50%.

Animal experiment. During the throughout feeding period of 4 weeks, no significant difference was observed

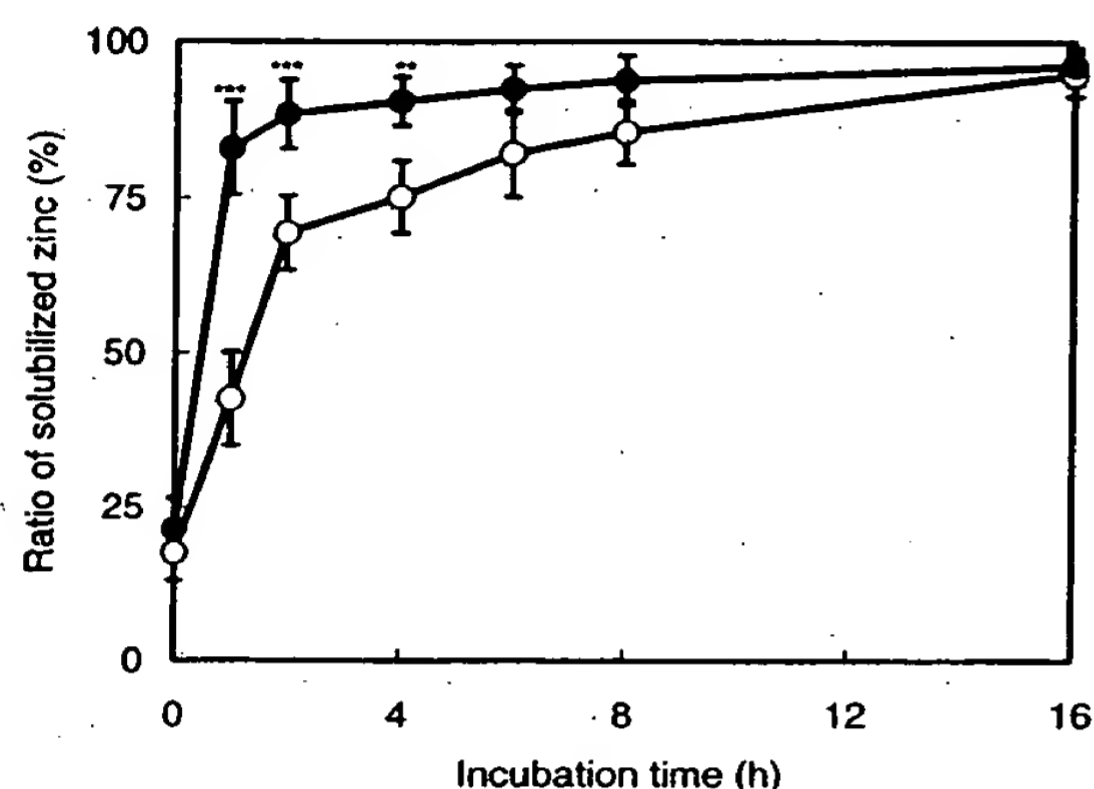


Fig. 1. Effect of the zinc source on zinc solubilization in experimental diets during trypsin digestion

Ten grams of each zinc-supplemented diet were mixed with 50 ml of 0.1N HCl containing 300 mg of a dried powder of porcine pepsin and incubated with shaking at 37 °C for 3 h. After pepsin digestion, the pH of the reaction mixture was adjusted to 7.4 by the dropwise addition of 1M NaOH. Then, 30 mg of crystalline porcine trypsin (5,600 USP trypsin unit/mg, Wako) was added to the mixture and incubated with shaking at 37 °C for a further 16 h. At each time, a portion of the reaction mixture was centrifuged at 6,000 x g for 30 min and the zinc in the supernatant was determined.

Vertical bars denote the SD of the mean for four determinations: those marked with asterisks differ significantly (Student's t-test) from the corresponding control value: ** $p < 0.01$, *** $p < 0.001$

(○) zinc carbonate hydroxide (●) zinc-rich powder of oyster

in the body weight or growth, irrespective of the dietary zinc status; at the end of the experimental period, the mean \pm standard deviation of body weight for all the rats ($n=18$) was 215 ± 12 g. Similarly, the effect of the dietary zinc status was least significant on tissue weight (data not shown).

The zinc deposition in the plasma, erythrocytes, liver, muscles, and tibia are summarized in Table 4. The zinc deposition in the blood was not increased by dietary zinc supplementation. The liver and muscle zinc tended to increase with the addition of dietary zinc regardless of its source. On the other hand, zinc in the tibia varied according to the zinc source. ZRP supplementation significantly increased the zinc deposition in the tibia, although supplementation with zinc carbonate hydroxide had no effect.

DISCUSSION

Since oysters contain zinc at an especially high level, the utilization of oysters as a component of zinc-rich food-stuffs or a zinc supplement is expected. However, hot water extract of oyster, which is usually used as a nutritional supplement in Japan does not contain zinc level enough for this purpose. The ZRP prepared in this study contained a high concentration of zinc at a level of 7.6%. The powder also contained sodium, phosphorous and calcium at a comparable level to zinc. However, the recommended dietary allowance or intake of these minerals is over 50 times higher than that of zinc [1, 7]. Thus, the utilization of ZRP as a zinc supplement does not influence the daily intake of these minerals quantitatively. In addition, the levels of magnesium, iron, copper and manganese were much lower than zinc. Accordingly, ZRP can be used as a nutritional supplement specifically increasing zinc intake.

Many approach have been used to investigate zinc absorption in foods. The *in vitro* approach to zinc absorption has been performed by several researchers [8-10].

Table 4. Zinc deposition in the tissue of rats fed experimental diets

Zinc added	Tissue zinc concentration ($\mu\text{g/g}$)				
	Plasma	Erythrocytes	Liver	Muscle	Tibia
None	0.29 ± 0.08^a	2.51 ± 0.11^a	20.8 ± 1.4^a	8.09 ± 0.59^a	13.2 ± 1.6^a
Zinc carbonate hydroxide	0.29 ± 0.07^a	2.41 ± 0.10^a	22.3 ± 0.7^{ab}	8.55 ± 0.41^a	11.8 ± 0.4^a
Zinc-rich powder of oysters	0.30 ± 0.07^a	2.55 ± 0.03^a	24.9 ± 0.7^b	8.42 ± 0.60^a	17.3 ± 1.0^b

Values are the means \pm SEM for 6 animals. Means in the same column not sharing a common superscript letter differ significantly ($p < 0.05$).

In these reports, solubility and chemical characteristics of zinc in an *in vitro* digest were used for an index of absorption. In the present study, ZRP released zinc more readily than zinc carbonate hydroxide by *in vitro* protease digestion of the zinc-supplemented experimental diets. Accordingly, it is implied that the solubility of zinc in ZRP was higher than zinc carbonate hydroxide in the small intestine.

In the animal experiment, the deposition of zinc from ZRP was significantly higher than that from zinc carbonate hydroxide in the tibia. This result is coincident with that of the *in vitro* digestion test. That is, higher solubility of the ZRP could cause a higher intestinal absorption of zinc from ZRP and resulted in the higher zinc deposition in the tibia of rats fed the ZRP-supplemented diet. Therefore, it can be concluded that the bioavailability of zinc in ZRP is superior to zinc carbonate hydroxide.

Since ZRP is insoluble at a neutral pH, some of the zinc may exist as a form of zinc hydroxide. Nevertheless, a difference between ZRP and zinc carbonate hydroxide was observed in the evaluation of bioavailability. It has been reported that several dietary factors influence zinc absorption [11]. In particular, zinc absorption is enhanced by several dietary ligands and chelators, including low-molecular-weight peptides or amino acids [12-14]. As described in Table 1, the main component of ZRP is acid-soluble Kjeldahl nitrogen, *i.e.* low-molecular-weight peptides and amino acids. These compounds may include ligands or chelators for zinc and promote zinc absorption.

ZRP contained zinc at a particularly high level and its bioavailability was satisfactory. Thus, the use of ZRP as a zinc supplement or for zinc enrichment of food is expected.

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Wako Pure Cl

* 321-25822 水素 Zinc Gluconate n-Hydrate グルコン酸	C ₁₂
* 325-25825 水素	
503-71551 AVO	
583-69285 ALF Zinc Hexaborate ヘキサほう酸亜鉛	Zn ₆
570-47851 SRM Zinc Hexafluoroacetylacetonate Di	
588-69291 ALF Zinc Hydroxide 水酸化亜鉛	Zn(OH) ₂
265-00312	Zn
267-00311	
269-00315	
268-01061	
266-01062	
589-69302 ALF Zinc Iron Oxide 酸化鉄亜鉛	ZnFe ₂ O ₄
266-00325	
325-27581 水素 Zinc Laurate ラウリン酸亜鉛	C ₁₂ H ₂₅ O ₂ Zn
588-69311 ALF Zinc Molybdenum Oxide 酸化モリブデン	ZnMoO ₄
322-27591 水素 Zinc Myristate ミリスチン酸亜鉛	C ₁₄ H ₂₇ O ₂ Zn
263-01136	
580-60471 ALF Zinc Neodecanoate ネオデカン酸亜鉛	C ₂₀ H ₃₉ O ₂ Zn
269-00332	
263-00335	
267-01031	
265-01032	
585-69321 ALF Zinc Nitride 窒化亜鉛	Zn ₃ N ₂